THE EFFECT OF OSMOTIC STRESS ON THE IONIC EXCHANGE OF A SHORE CRAB

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The decapod Crustacea have received considerable attention with regard to their ability to regulate the inorganic ions of their blood (Krogh, 1939; Robertson, 1949, 1953, 1957; Prosser *et al.*, 1950). Prosser *et al.* (1955) studied responses of the shore crab *Pachygrapsus crassipes* to different concentrations of sea water. The chief concern of their study was to determine the changes in the ionic concentrations of blood and urine which were effected by altering the concentration of the external medium from normal. Determinations on the total losses and gains of the respective ions between animal and medium were not made nor were the effects of desiccation on ion concentrations in urine or blood determined. This information would be of special interest in the case of a semi-terrestrial crab such as *Pachygrapsus*.

Gross (1958) demonstrated that when *Pachygrapsus crassipes* was placed under osmotic stress, the principal exchanges of potassium were between the medium and a source of potassium other than the blood, not mainly between blood and external medium. Also, evidence was produced that an extra-vascular pool participates in sodium exchanges between crab and medium. This paper will produce further evidence that extra-vascular salt pools in *Pachygrapsus* contribute to ionic exchanges with the medium, special attention being paid to calcium and magnesium. The effects of desiccation on the ionic concentration of urine and blood in *Pachygrapsus* will be revealed and data confirming the findings of Prosser *et al.* (1955) will be produced.

Material and Methods

The subject of this investigation, *Pachygrapsus crassipes* Randall, was collected at Laguna and Dana Point, California. All specimens were between molts, and were mature, none being smaller than 20 gm.

Urine was sampled by inserting a micropipette into the excretory pores. Blood was obtained by puncturing the cuticle of the leg joints with a micropipette. Sodium and potassium were measured by means of a Beckman flame photometer. Urine and blood were measured and diluted appropriately before being used directly in the flame photometer (Gross, 1958). Samples as small as 0.05 ml. thus could be analyzed to an accuracy of 2% for sodium and 10% for potassium at the minimum concentrations measured in this investigation. Thus, before and after treatment samples of blood from the same crab could be analyzed for sodium and potassium. Calcium and magnesium were determined by titration with ethylenediamine tetra acetic acid (EDTA) a method described by Schwarzenbach *et al.* (1946) and Knight (1951). This method requires about 0.25 ml. of urine and

about 0.50 ml. of blood. Because of the relatively large volume needed, repeated blood samples on the same specimen were not taken. Urine samples were diluted to 100 ml. and titrated directly. Media were titrated directly. Blood samples were dialyzed against distilled water and the dialysate was titrated, a correction being applied for the content of the dialysis bag. This process, which was not necessary in the case of urine, gave a more distinct end-point than titrating the diluted blood directly. Calcium and magnesium thus could be recovered within an accuracy of 5% for the minimum concentrations measured.

TABLE I

Effects of stress on ionic concentrations of blood and urine in Pachygrapsus

	Treatment											
	50% sea water			100% sea water			150% sea water			Desiccation		
	Mean	S.D.	No. crabs	Mean	S.D.	No. crabs	Mean	S.D.	No. crabs	Mean	S.D.	No. crabs
Sodium (mEq./l.)												
Blood	397	24	37	*483	17.3	36	582	34	30	536	27.4	32
Urine	380	60	37	378	64.0	15	353	106	30	297	104	15
U/B ratio	0.96	0.14	37	0.78	0.14	15	0.63	0.16	30	0.56	0.17	15
Medium	232			464			696					
Potassium (mEq./l.)												
Blood	7.36	1.4	37	*7.43	0.72	36	10.23	1.48	30	11.5	1.63	32
Urine	9.95	3.5	37	7.76	1.35	15	9.59	1.13	30	14.8	3.18	15
U/B ratio	1.45	0.50	37	0.82	0.19	15	0.94	0.33	30	1.34	0.50	15
Medium	4.9			9.8			14.7					
Calcium (mEq./l.)												
Blood	34.8	7.9	24	29.6	5.9	44	36.4	4.8	30	45.2	10.7	36
Urine	32.7	7.1	31	36.0	6.3	15	47.9	5.2	20	44.4	7.36	12
U/B ratio	0.98	0.13	23	1.17	0.20	15	1.33	0.18	29	1.07	0.33	12
Medium	10.0			20.0			30.0					
Magnesium (mEq./l.)												
Blood	13.6	5.36	24	20.0	6.1	44	27.1	4.22	30	28.5	15.9	36
Urine	70.5	41.1	31	236	87	15	408	122	29	424	144	12
U/B ratio	5.62	4.52	23	13.6	5.3	15	15.4	4.44	29	23.6	10.9	12
Medium	52.0			104			156					

* Gross (1958).

In order to measure the exchange of ions between animal and external medium, crabs freshly removed from normal sea water were weighed, and blood was sampled for sodium and potassium determinations. The crabs then were rinsed in water of the salinity to which they were to be exposed, then immersed in a small volume (50 ml.) of that same water for a period of about 24 hours. Adequate precautions were taken against water loss by evaporation. Values concerning sodium and potassium exchanges (Table II) have been reported previously (Gross, 1958)

and include some data on animals immersed 24–48 hours in 100 ml. The crabs could raise themselves out of the water and therefore were not completely immersed at all times. After a period of about 24 hours, the animals were removed from the media and their blood and urine sampled for the analysis of sodium, potassium, calcium and magnesium. Likewise the media were analyzed for these ions.

Other crabs freshly removed from normal sea water were weighed, then desiccated for a period of about 48 hours for a loss of about 7% original weight. After this treatment the blood and urine were analyzed for the above four cations.

TABLE II

Relative ion changes in blood and external medium caused by altering external medium from normal

	Mean of ratios*	50% sea water	S.D.	No. crabs	150% sea water	S.D.	No. crabs
Na	Blood change (mEq./l.) / Medium change (mEq./l.)	2.56	0.82	28	2.63	0.80	25
K	Blood change (mEq./l.) / Medium change (mEq./l.)	0.56	0.43	20	1.00	0.70	24
Ca	Blood change (mEq./l.)** / Medium change (mEq./l.)	0.93	3.23	22	0.78	0.66	28
Mg	Blood change (mEq./l.)** / Medium change (mEq./l.)	0.85	0.89	24	0.64	0.40	26

* Change in medium for all ions is corrected to a volume equal to the weight of the crab.

** Blood change for calcium and magnesium equals the difference between mean of normal crabs and the observed blood concentration after treatment for each crab. Medium change is the observed concentration change in the medium after treatment for each crab.

Analyses of blood potassium and sodium were made before and after desiccation on individual crabs.

RESULTS

Table I presents the urine and blood concentrations of sodium, potassium, calcium, and magnesium after the following treatments: a) immersion in normal sea water; b) immersion in 50% sea water; c) immersion in 150% sea water and d) desiccation for a water loss of about 7% body weight. Comparing the blood values after immersion in 100% sea water with those of Prosser *et al.* (1955), sodium and calcium appear in agreement. However, the potassium (7.43 mEq./l.) and magnesium (20.0 mEq./l.) values are considerably less than those reported by the above workers (12.1 mEq./l. and 58.4 mEq./l., respectively). On the other hand Schlatter (1941) reported blood ion concentrations for this same species which agree closely with the values of the present investigation.

It should be emphasized that the indicated stress media (Table I) represent only the initial sea water concentrations, and that these necessarily were altered by exchanges of salts with the animal. However, an accurate knowledge of the sustained osmotic gradient and the final blood concentrations is of little meaning in this investigation, since as described above, the animals were able to raise them-

selves out of the water. The main objectives of this study are to demonstrate: 1) the degree to which a blood ion change is reflected in the external medium and 2) the role of the antennary glands in controlling the ion content of the animal. It also should be pointed out that in this crab alterations in the blood concentration in aqueous media are effected by salt exchanges, not water (Gross, 1957).

Data in Table I, however, do reveal something of the ability of *Pachygrapsus* to regulate ions in the different sea water concentrations. Thus blood sodium is held above the sodium concentration of the dilute medium and normal sea water, but below the concentration of the hypertonic medium. Blood potassium is held above the concentration of the dilute medium, but below the concentration of normal sea water or the concentrated medium. Gross (1958) reported that when *Pachygrapsus* was immersed in a small volume of 50% sea water, the blood potassium remained less concentrated than the medium potassium. However, these animals were immersed for longer periods than those reported in the present studies (Table I) during which time the animal lost more potassium and the medium gained potassium. Table I also shows that the blood calcium remains more concentrated than the medium calcium for all treatments. Blood magnesium, on the other hand, is less concentrated than the medium magnesium for all aqueous conditions. All four ions increase under conditions of desiccation.

The ratios, urine concentration/blood concentration (U/B ratio), for each respective ion suggest the role of the antennary glands in the ion regulatory mechanism. Values in Table I are means of U/B ratios observed in individual specimens, not ratios of means. Thus all the mean U/B ratios for sodium are less than one, indicating that the antennary glands do not regulate sodium under this set of conditions. That is, sodium is not eliminated effectively when the gradient between blood and medium favors a gain; nor is it conserved effectively when the gradient favors a loss to the medium (mean U/B ratio in 50% sea water = 0.96).

With respect to potassium the mean U/B ratio is less than one when the crab is immersed in 100% or 150% sea water. Thus the antennary gland does not regulate potassium for this set of conditions. In 50% sea water the mean U/B ratio is 1.45 which means, if anything, potassium is being wasted when it is needed. However, for conditions of desiccation the mean U/B ratio is 1.34 which is significantly greater than one, $P < 0.01$. If then there were sufficient production of urine under conditions of desiccation, the antennary glands would tend to keep the blood concentration of potassium at a normal level.

With respect to calcium the mean U/B ratios for crabs immersed in 50% sea water or subjected to desiccation are not significantly different from unity. Thus the antennary glands are ineffective as regulators of calcium for these two conditions. On the other hand, after immersion in 150% sea water the mean U/B ratio is 1.32 which is significantly different from one, $P < 0.01$. In normal sea water the U/B ratio is 1.17, again being significantly greater than one, $P < 0.01$. Thus, the antennary glands might have a small role in regulating calcium, but in no sense as large a role as they have for magnesium.

Data in Table I demonstrate that the mean U/B ratios for magnesium under all conditions studied are much greater than unity. Even after immersion in 50% sea water, the mean ratio is 5.62. However, it should be pointed out that even in this diluted sea water the gradient between blood and external medium favors the

uptake of magnesium. Also, it will be noted that the mean ratio under conditions of desiccation is 23.6 which suggests that the urine concentration depends on the blood concentration, not entirely on the rate of influx from the external medium.

The data presented in Table I concerning the treatments in aqueous media are qualitatively in general agreement with the findings of Prosser *et al.* (1955), particularly with regard to the role of antennary glands in the regulation of magnesium. Quantitatively the data presented in Table I differ somewhat from those reported by Prosser *et al.* (1955). However, precise comparison should not be attempted because of differences in experimental procedure. For example, crabs of the present investigation were immersed directly in small volumes of stress media for a maximum of about 24 hours. The data presented by the above workers were obtained on animals gradually acclimated to osmotic stresses for a period of at least 5 days in relatively large volumes of media.

On the other hand there are certain differences which warrant attention. Normal blood potassium and magnesium differences already have been mentioned above. It will be observed that blood calcium after immersion of the animal in 50% sea water (34.8 mEq./l.) is higher than it is for animals from normal sea water (29.6 mEq./l.). These means are significantly different; $P = 0.01$. Prosser *et al.* (1955) showed decreases in blood calcium in 50% sea water which, of course, would be expected. It was thought that perhaps the increased blood calcium resulting from immersion in dilute sea water was an effect of the small volume of medium. Therefore, blood calcium of crabs immersed in large volumes (about 700 ml.) of 50% sea water for 24 hours was determined. The mean blood calcium of 24 crabs thus treated was 30.9 mEq./l., S.D. = 9.0. This is not significantly different from the mean (34.8) obtained by the other treatment; nor is it significantly different from the average blood calcium of normal crabs. These workers also called attention to the inverse relationship between the urine sodium concentration and the blood sodium concentration. That is, the urine sodium of animals immersed in concentrated sea water was less concentrated than that of animals immersed in normal sea water, which in turn was less concentrated than that of animals immersed in dilute sea water. The means for urine sodium after treatment in the three aqueous media (Table I) cannot be shown to be significantly different, but the U/B ratios do suggest the same phenomenon. That is, the ratios decrease as the animal is placed in increasing concentrations of sea water. These ratios are all significantly different from each other; $P < 0.01$. The U/B ratio for the desiccated crabs is not significantly different from the U/B ratio in crabs exposed to concentrated media, but is significantly different from the ratios obtained for crabs given the other treatments; $P < 0.01$.

Data in Table II demonstrate the ionic changes that occur in the medium when a given change in the blood is effected. The measurement of calcium exchanges with stress media was complicated by the fact that this ion is lost in significant amounts when the animal is immersed in normal sea water. Such was not the case for the other ions. It became necessary, therefore, to apply a correction to the calcium exchanges, based on an average loss to normal sea water by 30 crabs. This amounted to 0.5 mEq./l. per gram of crab for a 24-hour period in 50 ml. of medium. It was thus necessary to assume that this *normal* loss is constant in all concentrations of sea water, an assumption which subjects the values for calcium change in the medium to considerable error.

The values for sodium and potassium have been reported previously (Gross, 1958) and represent means of the ratios, blood change (mEq./l.)/medium change (mEq./l.), in individual crabs where the blood change is the difference between the concentration before treatment and the concentration after treatment. For calcium and magnesium the values in Table II also represent means of the ratios, blood change (mEq./l.)/medium change (mEq./l.), in individual crabs, but since only one sample of blood could be extracted from single specimens for calcium and magnesium determinations, the blood change (mEq./l.) in the ratio for calcium and magnesium equals the difference between the observed blood concentration after treatment and the average blood concentration for crabs from normal sea water.

With respect to sodium, the mean ratios are greater than 2.5 in both 50% and 150% sea water. The response to hypertonic stress and hypotonic stress seems to be symmetrical. With respect to potassium the ratio is unity or less; while it is 0.56 for crabs immersed in 50% sea water, it is 1.00 for crabs immersed in 150% sea water. However when ion exchanges were measured in crabs transferred from 50% to 150% sea water or vice versa, a symmetrical response for potassium

TABLE III

Ion increase in blood caused by desiccation

	No. crabs	Mean change in concentration (% original) per 1% body weight loss by evaporation	S.D.
Na	84	+2.20	0.71
K	50	+8.68	11.75
Ca	34	+5.47	4.23
Mg	35	+3.87	9.42

exchanges is observed, the mean ratio, change in blood (mEq./l.)/change in medium (mEq./l.), being about unity in both extreme stresses (Gross, 1958).

The mean ratio for calcium and magnesium is less than one for all treatments. Attention should be called to the large variance for the calcium ratio, following immersion in 50% sea water. It also should be mentioned that the ratio, mean blood change (mEq./l.)/mean medium change (mEq./l.), is $\frac{+5.2}{-1.82} = 2.87$, the signs of the numerator and denominator being opposite to expectation. Not only does the average value for the blood calcium increase after treatment in dilute sea water, but the medium apparently loses rather than gains calcium. The difference between the mean of the ratios (0.93) and the ratio of the means (2.87) can be explained on the basis of the large variance.

Table III reveals ionic changes that occur in the blood when *Pachygrapsus* is desiccated for a loss of about 7% body weight. The sodium and potassium values, again, have been reported previously (Gross, 1958) and represent averages of changes in individual crabs, where the blood concentration change was determined by before- and after-treatment readings on the same individual. The values for calcium and magnesium are means of blood concentration changes for individual

crabs, but since only after-treatment blood samples were taken, the blood change for these two ions is represented by the difference between the observed concentration in an animal following desiccation and the mean blood concentration of the respective ions in crabs from normal sea water. In Table III it can be seen that the average change for sodium is less than the values for the other ions. While the potassium and calcium changes are significantly greater than the sodium change, $P < 0.001$, the mean magnesium change cannot be considered significantly different from the sodium change. It will be explained below that blood ions which increase more in concentration than blood sodium probably shift from a salt pool (perhaps the intra-cellular space) into the blood when the animal is desiccated.

Discussion

The ratios, blood change (mEq./l.)/medium change (mEq./l.), presented in Table II suggest that the principal exchanges of potassium, calcium, and magnesium between animal and medium are not ultimately between blood and external medium. A ratio of unity means that the concentration change in an external medium which is equal in volume to the animal is identical to the concentration change in the blood. Of course, much of the animal's volume is isolated from the osmotic and ionic processes which occur in the blood. Thus for a ratio of unity, the actual loss or gain of ions with the medium would be greater than the loss or gain of ions in the blood. Therefore a source other than the blood must be contributing to these exchanges. These ratios also can be expressed as "apparent volume of distribution," using the equation $V = M/P \times 100$ (Gross, 1958) where:

V = "apparent volume of distribution" in % body weight;

$$M = \frac{\text{weight of medium}}{\text{weight of animal}};$$

$$P = \frac{\text{change in blood ion concentration (mEq./l.)}}{\text{change in medium ion concentration (mEq./l.)}}.$$

Thus, the "apparent volume of distribution" for sodium is 38.5% body weight and for potassium, calcium and magnesium more than 100% body weight, which only can be interpreted as an aggregation of these three ions in some sort of pool where they are much more concentrated than they are in the blood. This also means that the extra-vascular pools ultimately contribute more to potassium, calcium and magnesium exchanges with the medium than does the original blood supply (more than twice as much). At least, in the case of potassium, the pool probably lies mainly in the intra-cellular space, because it is well known that intra-cellular potassium concentrations are high. In the crab *Carcinus* the relative muscle concentrations of sodium, potassium, calcium and magnesium are 50, 120, 11 and 32 (mEq./kg. water), respectively (Shaw, 1955). If this were representative of intra-cellular concentrations, it would seem unlikely that the intra-cellular space harbors the pool for magnesium and calcium. Although the nature of the pools is unknown, it becomes apparent that a change of a blood ion concentration can occur without a loss or gain in the medium. Or exchanges between animal and medium can occur without being reflected in the blood. The probable exception to

this is sodium. The "apparent volume of distribution" for sodium was calculated to be 38.5% body weight for the moderate stresses of 50% and 150% sea water. Webb (1940) estimates the blood volume of the crab *Carcinus* as 36% body weight. Thus the calculated volume, 38.5% body weight, which seems close to a reasonable value for blood space, means that the major sodium exchanges are between the blood and external medium. Though there is evidence that a sodium pool contributes to such exchanges when the animal is exposed to extreme osmotic stress, its role is relatively small percentage-wise, compared with the other ions (Gross, 1958). On the other hand sodium contributes about half the ions of the blood; thus the small percentage effect of a sodium pool would nevertheless affect significantly the total osmotic pressure of the blood.

Burger (1957) immersed lobsters in media of abnormally high magnesium concentrations and noted that neither the blood nor the urine magnesium elevated. On this evidence he concluded that the animal was impermeable to magnesium. However, he did not consider the possibility that the magnesium could enter the animal and be fixed outside of the vascular system, a phenomenon which obviously occurs in *Pachygrapsus*.

The variance for the mean of the calcium ratios, blood change/medium change, when the stress was 50% sea water is high. Nevertheless this ratio for calcium (0.93) is significantly less than the mean ratio for sodium (2.56), $P < .025$. It should be emphasized that the mean blood calcium after immersion in 50% sea water was more concentrated than that for crabs from normal sea water. Also, the corrected average change for calcium in the medium indicated a loss rather than the expected gain. Now, it was revealed above that crabs in normal sea water tend to lose calcium, and the average loss in normal sea water was applied as a correction to the medium measurements, assuming that a loss of calcium (probably by way of the gut) would be the same in a stress as in a normal medium, but if there were a curtailment of *normal* calcium output in dilute sea water, then the correction would be too large and falsely could make the sign of the change in the medium negative. It should be mentioned that the observed changes in the medium without correction were all positive. If the sign of the corrected medium change is in error, then the increase in the blood calcium concentration after immersion in 50% sea water could be caused only by contributions from a calcium reservoir.

Data in Table III demonstrate that for a given weight loss by evaporation the average increase in the blood sodium concentration is less percentage-wise than the increase for the other ions. It was concluded by Gross (1958) that such a difference in increase between sodium and potassium under conditions of desiccation could not be explained on the basis of sodium exclusion from the blood. Rather, it was concluded that it represented a shift of potassium ions from extra-vascular spaces into the blood space. Data for calcium presented in Table III suggest that the same phenomenon happens in the case of this ion; values for magnesium are questionable. No adaptive significance can be assigned to such a phenomenon; rather it is interpreted as a physiological failure which imposes a limitation on the terrestrial habits of this crab.

The U/B ratios presented in Table I suggest the role of the antennary gland as an ion regulator. It has been established previously (Prosser *et al.*, 1955)

that this organ is ineffective as an osmotic regulator. Thus, it seems probable that a principal function of the antennary gland is the regulation of magnesium. That is, the U/B ratio with respect to magnesium is much greater than unity. Yet the effectiveness of the antennary glands as magnesium regulators for each experimental condition cannot be known for certain until the volume of urine production is known for each osmotic situation. Thus, even though the urine magnesium is high when the animal is desiccated, it is possible that little or no urine is produced when the animal is removed from an aqueous medium. Nevertheless, the antennary glands may effectively remove magnesium ions from the blood, thus tending to keep the blood levels normal, even though no ions are ejected from the animal.

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Summary

1. The effects of osmotic stress on the ion concentration in the blood of the crab, *Pachygrapsus crassipes*, were investigated. Stresses imposed were 50% sea water, 150% sea water and desiccation to a water loss of about 7% body weight.

2. The observed ratios, blood change (mEq./l.)/medium change (mEq./l.), for sodium, potassium, calcium and magnesium after the crab was transferred from normal sea water to 50% or 150% sea water yielded values for "apparent volume of distribution." The average value for sodium was 38.5% body weight, but for the other three ions was at least 100% body weight.

3. The large values for "apparent volume of distribution" in the cases of potassium, calcium and magnesium indicate that these ions are contained in extravascular pools in greater concentrations than they are in the blood and that these pools participate in ion exchanges between animal and medium. Thus, a concentration change can occur in the blood without being reflected in the medium or vice versa.

4. Calcium is lost to the medium by *Pachygrapsus* when it is immersed in normal sea water. Blood calcium increases when a crab is transferred from normal sea water to dilute sea water.

5. When *Pachygrapsus* is desiccated, the blood concentrations of potassium, calcium and magnesium average greater increases than does the sodium concentration. This suggests that potassium, calcium and possibly magnesium shift from an extra-vascular pool into the blood space. The phenomenon is interpreted as a physiological failure and a factor which may limit the terrestrial life of this species.

6. The ratio, urine concentration (mEq./l.)/blood concentration (mEq./l.), for the respective ions suggests the role of the antennary glands as ion regulators

under the various stress conditions. Thus the antennary glands were found to be relatively ineffective as regulators of sodium, potassium and calcium for all conditions studied. The U/B ratio for magnesium averaged 5.62 when the crab was immersed in 50% sea water; 13.6 for normal sea water; 15.4 for 150% sea water and 23.6 when the crab was desiccated. These high ratios suggest that a principal role of the antennary glands is magnesium regulation.

7. The volumes of urine production which have not been measured must be known before the effectiveness of the antennary glands as magnesium regulators can be determined.

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